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<b>(21) International Application Number:</b> PCT/IB96/01083 <b>(22) International Filing Date:</b> 11 October 1996 (11.10.96)  <b>(30) Priority Data:</b> 60/005,585                      12 October 1995 (12.10.95)      US Not furnished                11 October 1996 (11.10.96)      US  <b>(60) Parent Application or Grant</b> (63) Related by Continuation US    60/005,585 (CON) Filed on                                      12 October 1995 (12.10.95)  <b>(71) Applicant (for all designated States except US):</b> CHIRON DIAGNOSTICS CORPORATION [US/US]; 333 Coney Street, East Walpole, MA 02032 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> WALKER, Roger, P. [US/US]; 861 Oxford Way, Benicia, CA 94510 (US). EL- AHMADY, Omar [EG/EG]; 2 Roshdy Street, Safeer Square, Heliopolis, Cairo (EG).  <b>(74) Agent:</b> MORGENSTERN, Arthur, S.; Ciba Coming Diagnos- tics Corp., 63 North Street, Medfield, MA 02052 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title: DETERMINATION AND MONITORING OF BLADDER CANCER</b>		
<b>(57) Abstract</b>  A test method for determination of the presence of bladder cancer by determination of the urinary gonadotropin peptide (UGP) levels in no-blood body fluids is described. The method can also be used to monitor the course of bladder cancer and its treatment and the reoccurrence of the disease.		

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## DETERMINATION AND MONITORING OF BLADDER CANCER

### Field of the Invention

The present invention is directed to methods of determining the presence or  
15 absence of bladder cancer in individuals as well as methods of monitoring the  
effectiveness of the methodology for treating bladder cancer and for monitoring the  
course of bladder cancer in the body of an individual. Urinary gonadotropin peptide,  
a core fragment of the beta subunit of human chorionic gonadotropin (hCG) is used as  
the marker to distinguish bladder cancer as opposed to urogenital benign disease or  
20 normalcy and to allow monitoring of the course of bladder cancer before or after  
treatment of the cancer.

### Background of the Invention

Bladder cancer has a high incidence throughout the world and in some  
25 countries as, for example Egypt, it is the most common type of male malignancy; and  
in females, ranks only after breast cancer in rate of incidence. The disease, as known  
in the art, is characterized in the Mideast and Far East by a high predominance of  
locally advanced lesions, and a high incidence of squamous cell carcinoma, *Khaled,*  
*H.M. 1993, The Cancer J., 6,65-71 Bladder Cancer and Bilharziasis Today.* In

industrialized countries, the predominant form of bladder cancer is transitional cell carcinoma, and 70% of these cases are superficial, noninvasive types.

Thus, the pathology of bladder cancer can vary as a function of the specific population, and regardless of the histologic type, the main prognostic factors are stage, grade, and spread of the tumor to tissues outside the bladder. Despite the differences in the natural history of bladder cancer as a function of geography and/or population, tumor markers can be used to aid in the management of disease. At least initially, for each population, the range of values of the tumor marker for normal individuals and patients with benign disease must be determined in order to allow determination of a cutoff that distinguishes patients with malignancies. Generally, a cutoff value for a tumor marker below which 95 percent of normal and benign subjects fall is chosen as the dividing point between normalcy and malignancy. This 95 percent confidence interval is useful for comparison of the sensitivity of various tumor markers in detecting malignant disease.

A variety of tumor markers have been evaluated for detecting bladder cancer with varying results and terms of sensitivity and specificity. Tissue polypeptide antigen (TPA) has been one of the most reliable markers and along with the use of carcinoembryonic antigen (CEA) and ferritin with TPA has increased the diagnostic value of TPA in detecting bladder cancer. Very recently, urinary levels of human chorionic gonadotropin beta subunit (beta-hCG) have been evaluated in Egyptian bladder cancer patients and patients with benign urinary tract disorders. This marker was elevated in 60.3% of cancer patients, however, 29.7% of patients with benign disease were also elevated above the limits of the normal control group and thus prior studies did not determine that beta-hCG would be useful as a method of determining or monitoring of bladder cancer in individuals. In another study, elevated levels of serum beta-hCG have been shown to occur in 47% of patients with transitional cell bladder cancer (I. Marcillac *et al.*, Free Human Chorionic Gonadotropin beta subunit in Gonadal and Nongonadal Neoplasms. Cancer Research, 52, 3901-3907, 1992). In another study, human chorionic gonadotropin, beta hCG, and beta-core fragment were demonstrated to be the predominant species present in the serum and urine of several

patients with metastatic transitional cell carcinoma (R.K.Iles et al., Composition of intact hormone and free subunits in the human chorionic gonadotropin-like material found in serum and urine of patients with carcinoma of the bladder. Clinical Endocrinology, 32, 355-364, 1990).

5 U.S. Patent 5,356,817 issued October 18, 1994, entitled METHODS FOR  
DETECTING THE ONSET, PROGRESSION, AND REGRESSION OF  
GYNECOLOGICAL CANCERS, suggested the use of human chorionic gonadotropin  
beta-subunit core fragment (which is different from HCG) for detecting the  
progression or regression of gynecological cancers in females. Thus, this marker is  
10 known as a marker for certain gynecological cancers and is known for gynecological  
cancer detection in women by monitoring of non-blood body fluids such as urine. It  
is often not used as a marker for determining cancer in some cases because the levels  
determined from blood body fluids are not sufficient for accurate determinations. The  
marker is known to be a major component of pregnancy urine and to occur in the  
15 urine of patients with a variety of non-trophoblastic tumors including colorectal  
cancer, pancreatic and biliary cancer, gastric cancer and lung cancer. Studies have  
demonstrated it to be expressed by a wide variety of tumor tissues. The marker is  
expressed in a stage dependent manner in the urine of patients with cervical cancer,  
endometrial cancer, vulvar cancer and ovarian cancer.

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#### Summary of the Invention

It is an object of the present invention to provide methods of determining presence or absence of bladder cancer in individuals with good specificity and sensitivities for the detection of malignant disease.

25 Another object of this invention is to provide methods for monitoring the course of bladder cancer in the body and/or determining the effectiveness of a methodology for treating bladder cancer.

Still another object of this invention is to provide methods in accordance with the preceding objects which are non-invasive and can be carried out with the use of

non-blood body fluids, including urine with minimum inconvenience and with good accuracy.

According to the invention, a test method of determining presence or absence of bladder cancer in an individual comprises obtaining a non-blood body fluid sample  
5 from an individual and then determining the urinary gonadotropin peptide (UGP) levels in the body fluid of the sample with elevated levels above normal indicating a cancer of the bladder and normal levels indicating the absence of cancer. Preferably, the body fluid is urine and conventional assay techniques are used to detect and quantify the amounts of urinary gonadotropin peptide (UGP).

10 In another method of this invention, an individual is monitored for the effectiveness of the methodology for treating bladder cancer in the body of the individual known to have bladder cancer. According to the method, in a first step, the individual is treated with any known methodology for reducing or treating bladder cancer. In the next step, the UGP level in a non-blood body fluid of the individual is  
15 monitored to indicate the measure of success of the treatment. Preferably, prior to the treatment for cancer, a non-blood body fluid sample is tested to determine the first UGP level and then testing is carried out after treatment to determine subsequent UGP levels. A drop in UGP level when measured successively over a period of time after treatment indicates some degree of success for the treatment. A rise in level would  
20 indicate the recurrence or growth of the cancer, while maintenance of normal values would be encouraging for success of the treatment.

In still another method, a person known to have bladder cancer either as a result of the above tests, and/or other medical procedures can be monitored for the course of the bladder cancer. The recurrence of bladder cancer after successful  
25 treatment can also be determined. UGP levels are determined from the urine or other non-blood body fluid over a period of time with variations noting the rise and lowering of cancer activity within the body.

It is a feature of this invention that standard assay techniques can be used in known methods to monitor UGP levels. Another feature of this invention includes the  
30 establishment of a positive level of UGP above which one can distinguish bladder

cancer from benign urological disease which level is preferably a level above 1.4 fmol/ml, but can be as low as above 0.7 fmol/ml. The testing of this invention for UGP levels can be carried out non-invasively with minimum discomfort to the patient or individual being tested. A single test can indicate elevated levels of concern, whereas prolonged testing over a period of time can be used for monitoring the individual and/or monitoring the effectiveness of a standard bladder cancer treatment. The urine can be tested immediately after withdrawal from the body or at substantial time periods thereafter while still maintaining good sensitivity and accuracy of the test.

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#### Brief Description of the Drawing

The above and other objects, advantages of the present invention will be better understood from a reading of the following specification in conjunction with the accompanying drawing in which the Figure illustrates the distribution of UGP values in normal subjects, patients with benign urological disease, and patients with bladder cancer. The number of subjects and mean UGP level (fmol/ml) for each group are indicated. The 95% confidence intervals for each population mean are indicated by the diamonds.

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#### Description of Preferred Embodiments

The methods of this invention call for the testing of UGP as a marker for the presence or absence of bladder cancer in a living mammal, preferably man or animals. UGP or urinary gonadotropin peptide is also known as urinary gonadotropin fragment (UGF), human chorionic gonadotropin beta-subunit core fragment and beta-core fragment. UGP is a 10.5 kilodalton glycoprotein with a primary sequence identical to residues 6-40 and 52-92 of the beta-subunit of human chorionic gonadotropin (hCG) as reported by Birken, S. et. al *Endocrinol.* 123, 572-583 (1988), *The Structure of Human Chorionic Gonadotropin Beta Core Fragment from Pregnancy Urine*. As is

30

known, the carbohydrate moieties of UGP differ significantly from hCG, lacking all O-linked species, and retaining only the core mannose, *N-Acetylglucosamine*, and *fucose residues* Blithe, DL, et. al, *Endocrinol.* 125, 2267-2272 (1989), *Carbohydrate Composition of Beta Core*. As used herein, UGP shall mean urinary gonadotropin polypeptide as described above.

UGP is measured in urine and can be measured in non-blood body fluids. Urine is preferred because of its ease of collection in a non-invasive technique in the body. UGP is highly stable in urine, and studies with pregnancy urine have indicated that samples can be stored at 4° C or 25° C for 21 days, or at -20°C for six months. Preservatives are not required to maintain clinical sample stability. UGP is not readily measured in serum due to its rapid clearance rate from the circulation. It is a major component of pregnancy urine and when pregnant individuals are tested, the test may not be accurate for determination of bladder cancer unless pregnancy determinations are also carried out. If pregnancy is detected, the instant test cannot be used as an indicator of UGP.

If elevated UGP were found, up until now there was only a concern for identifying gynecological cancers. However, now, if elevated UGP is found, the physician should investigate the presence of both gynecological cancers and bladder cancer.

The tests of this invention are sufficient to evaluate the expression of UGP in preoperative and postoperative patients with invasive bladder cancer and/or benign urogenital disease and in normal individuals in order to determine levels of UGP for use in the management of malignancy if present.

The testing to determine the UGP levels can be by conventional assay techniques. As an example, one assay can involve the determination of levels of UGP by determining the levels of hCG beta fragment and beta subunit and then testing for the C-terminal peptide (which is not present on the fragment). A preferred assay involves the use of a monoclonal or polyclonal antibody that recognizes any of hCG beta-fragment or beta-subunit. Known assays useful in this invention include Triton®



UGP EIA kits available from CIBA Corning Diagnostic Corp. of Alameda, California for the testing and quantitative measurement of UGP in urine.

The Triton® UGP EIA kit is a two-site enzyme immunoassay utilizing a specific monoclonal capture antibody and an enzyme-label polyclonal antibody  
5 directed towards different antigenic sites on the molecule.

Polystyrene tubes coated with mouse anti-UGP are incubated with urine specimens or the appropriate Calibrator or Control. During this incubation, the UGP molecules present in the specimen, calibrator and control are bound by the antibody onto the solid phase. Unbound materials present in the specimen are removed by  
10 washing of the tubes. In the second step, polyclonal anti-UGP Conjugated with horseradish peroxidase is added to the tube. If UGP molecules are present in the specimen, the Anti-UGP Conjugate is bound to the UGP on the tubes. Unbound conjugate is removed by tube washing. The tubes are next incubated with a TMB Substrate Solution (hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine) to develop  
15 a color which is a measure of the amount of bound Anti-UGP Conjugate. The intensity of the color developed is read with a spectrophotometer set at 450 nm. Color intensity is proportional to the concentration of UGP in the specimen, within the working range of the assay. A Calibration Curve is obtained by plotting the UGP concentration of the Calibrators versus the absorbance. The UGP concentration of the  
20 specimen and Control, run concurrently with the Calibrators, can be determined from the Calibration Curve. Since the concentration of urine analytes can vary between samples, creatinine levels in the urine can be used to compensate for this variation. In this example, the UGP values are divided by their respective creatinine values to give normalized values (fmol UGP/mg or mmol creatinine). Alternatively, 24 hour urine  
25 samples can be used or urines can be normalized by other factors, such as specific gravity.

In a specific example of the present invention, 450 individuals were classified into three groups and testing was carried out to determine presence or absence of bladder cancer.

The present study included 450 individuals classified into three groups. The first group included 237 patients with urinary bladder cancer that were admitted to the Egyptian National Cancer Institute. This group consisted of 171 males and 66 females ranging in age from 24 to 70 years. Lymph node involvement was present in 32 patients, and absent in 205 patients. Histopathological examination of the tumor tissues indicated 134 squamous cell carcinomas, 83 transitional cell carcinomas, 10 adenocarcinomas, 2 verrucous carcinomas, 2 leiomyosarcomas, and 6 undifferentiated carcinomas. As a function of stage 14 patients were stage T I and T II, 179 patients were stage T III, and 44 patients were stage T IV. When stratified by grade, 41 patients were grade 1, 118 patients were grade 2, and 78 patients were grade 3. Bilharzial ova were identified in 143 tumors, and absent in 94 tumors. Staging and grading were conducted according to the established TNM and WHO systems, respectively.

The second group consisted of 97 patients with benign urinary tract disease recruited from the urology outpatient clinic, Kasr El-Aini Hospital, Egypt, and included 90 males and 7 females ranging in age from 20 to 63 years. The benign disease categories includes 83 patients with urinary tract bilharziasis, and 14 with other benign disorders including benign prostatic hyperplasia, renal stones, varicocele, and bladder ulcers. The third group included 116 normal healthy controls who were free of disease as evidenced by clinical and laboratory investigations. This group consisted of 107 males and 9 females ranging in age from 20 to 52 years that were recruited from students and workers at Al-Azhar University, Cairo, Egypt.

All individuals were requested to collect 24-hour urine. Approximately 10 ml of each urine sample was centrifuged at 2000-3000 x g for 10 minutes, and the supernatant was frozen at -80°C until analyzed.

Urine collection was not limited to 24-hour samples. For example, a comparison of UGP levels in morning urine samples and 24-hour samples from 151 females was made and a significant correlation was found between the two types of samples ( $r=0.934$ ).

Urinary gonadotropin peptide (UGP) was determined in freshly-thawed urine samples. UGP was measured using an enzyme-linked immunoassay (*Triton* UGP EIA, Ciba Corning Diagnostics, Alameda, California). The *Triton* UGP EIA is a double-determinant enzyme immunoassay, which utilizes a monoclonal capture  
5 antibody immobilized on a coated tube, and an affinity-purified polyclonal antibody conjugated with horseradish peroxidase as the detection antibody. The assay has a minimum detectable concentration of 0.1 fmol/ml. Recovery of known quantities of UGP spiked into urine samples ranges from 86 to 109%, with a mean of 96%. The intra- and interassay reproducibility range from 4.12% to 4.95%, and 6.07 to 7.85%,  
10 respectively, over the range of the assay. Pathological urine samples exhibited linear dilution response, with a mean correlation coefficient of 0.999.

The assay is highly specific for UGP, exhibiting the following molar cross-reactivities: human chorionic gonadotropin (hCG, 0.11%), hCG beta subunit (0.043%), hCG alpha subunit (0.009%, human luteinizing hormone (hLH, 0.001%),  
15 hLH beta subunit (0.005%), human thyroid stimulating hormone and beta subunit (hTSH and hFSH-beta subunit, <0.001%), and human follicle stimulating hormone and beta subunit (hFSH and hFSH-beta subunit, <0.001%). The assay has been optimized to eliminate cross-reactivity with fragments derived from luteinizing hormone that are present in urine. The following urinary analytes do not interfere  
20 with the assay at levels up to the following concentrations: urea (5 g/dL), uric acid (150 mg/dL), creatinine (500 mg/dL), creatine (200 mg/dL), vitamin C (500 mg/dL), urobilin (4 mg/dL), glucose (30 mg/dL), and hemoglobin (10 mg/dL). The acceptable pH range of urine samples is from about 5.5 to about 8.5.

Further variations will be apparent to those with ordinary skill in the art. The  
25 following examples illustrate various aspects of the invention but are not intended to limit its usefulness.

#### Example 1

UGP levels were determined in 450 timed 24-hour urine samples from normal  
30 individuals, subjects with benign urological disease, and subjects with invasive

bladder cancer. The normal, benign disease control, and cancer patient cohorts were predominantly male, consisting of 107 (92%), 90 (93%), and 171 (72%) male subjects, respectively. The distribution of UGP values in these subject categories is shown in the Figure. UGP values are reported in units of fmol/ml in the 24-hour urine samples. Statistical analyses were performed using JMP software (SAS Institute). Population means were compared using the Tukey-Kramer HSD method. The mean UGP level in the bladder cancer patients was 4.86 fmol/ml, compared with 0.06 fmol/ml in the normal subjects, and 0.11 fmol/ml in the benign urological disease patients. The mean UGP levels in these populations were significantly different ( $p < 0.01$ ).

In order to evaluate the clinical performance of the UGP assay in distinguishing malignant disease from benign disease and normal individuals in this population, two cutoffs were used. The first cutoff was 0.7 fmol/ml, which was the calculated 95% specificity level based on two standard deviations above the mean UGP level of the benign disease population. The second cutoff was 1.4 fmol/ml, which was the 100% specificity level based on the distribution of UGP values in the same population. Using these cutoffs, the epidemiological sensitivity of UGP for detecting bladder cancer was evaluated according to various clinical parameters.

Table I shows the expression of UGP in 116 normal subjects, and 97 patients with benign urological disease. The majority of disease control patients ( $N = 83, 86\%$ ) had benign urinary bilharziasis. Mean UGP levels in the normal and disease control populations were similar, and ranged from 0 to 0.13 fmol/ml. Less than one percent of normal individuals, and six percent of patients with benign disease had UGP levels exceeding the 0.7 fmol/ml cutoff. The benign bilharziasis group showed the greatest number of patients exceeding the 0.7 fmol/ml cutoff, at 7.2%. None of the patients exceeded the 1.4 fmol/ml cutoff.

Table II shows the expression of UGP in bladder cancer patients as a function of histologic type of disease. The mean UGP value for all patients was 4.86 fmol/ml. Patients with squamous cell carcinoma (SCC) and transitional cell carcinoma (TCC) had the highest mean UGP levels of 4.84, and 5.40 fmol/ml, respectively. Patients

with other histologic types of malignant disease, including adenocarcinoma, undifferentiated carcinoma, verrucous carcinoma, and leiomyosarcoma had a mean UGP level of 2.76 fmol/ml. The differences in the mean UGP levels between these histotypes were not statistically significant ( $p > 0.05$ ). The percent of patients  
5 exceeding both cutoffs was similar for the TCC and SCC patients, for example, 65% of SCC patients and 71% of TCC patients exceeded the cutoff of 0.7 fmol/ml. Patients with other histologic types of disease exceeded both cutoffs in 50% of all cases.

Analysis of bladder cancer patients according to stage of disease is shown in  
10 Table III. An increase in UGP values from 3.22 fmol/ml for stage T I and T II patients, to 4.64 fmol/ml for stage T III patients, to 6.24 fmol/ml for stage IV patients was observed. Despite the apparent trend of increasing mean UGP level with advancing stage, these mean values were not significantly different ( $p > 0.05$ ). Similarly, the percent of patients exceeding the cutoff levels increased as a function of  
15 stage. At the 0.7 fmol/ml cutoff, 64% of stage T I and T II patients, 71% of patients with stage T III disease, and 81% of stage T IV patients exceeding the cutoff. The number of patients exceeding the 1.4 fmol/ml cutoff followed the same trend, but was correspondingly lower, ranging from 57% of stage T I and T II patients, to 73% of stage T IV patients.

20 When bladder cancer patients were stratified according to grade of disease (Table IV), mean UGP levels were lowest for grade 1 patients, and higher, but similar for grade 2 and 3 patients. Grade 1 patients had a mean UGP level of 2.93 fmol/ml, and grade 2 and 3 patients had mean UGP levels of 5.67 and 4.66 fmol/ml, respectively. The differences in mean UGP levels were significant only between the  
25 grade 1 and the combination of grade 2 and 3 disease ( $p < 0.05$ ). Overexpression of UGP values was similar for all grades at a cutoff of 0.7 fmol/ml, with 66% of grade 1 patients, and 75% and 73%, respectively of grade 2 and 3 patients exceeding the cutoff. At the higher cutoff of 1.4 fmol/ml, the percentage of patients with grade 1 disease exceeding the cutoff was 44%, which was significantly lower than the grade 2  
30 (66%) and grade 3 (59%) patients.

Stratification of bladder cancer patients according to nodal status and the presence of bilharzial ova in the tumor tissue is shown in Table V. For both categories, mean UGP levels in negative and positive cases were virtually identical to each other and to the mean value for all cancer patients, ranging from 4.82 to 4.88 fmol/ml. Similarly, overexpression rates at both cutoffs were virtually identical to each other and to the value for all cancer patients, ranging from 72-73% at the 0.7 fmol/ml cutoff, and 58-62% for the 1.4 fmol/ml cutoff. Finally, stratification of bladder cancer patients according to gender showed no difference in mean UGP levels. See data in Table VI.)

The above example demonstrated that UGP is also overexpressed in a majority of patients with invasive bladder cancer.

In this study population, UGP was demonstrated to be a sensitive and specific marker for malignancy. UGP was only marginally elevated in samples from normal individuals, and in patients with benign urogenital disease. Mean UGP levels in patients with bladder cancer were 81 and 44-fold higher than those in normal individuals, and patients with benign disease, respectively. At the 95% and 100% specificity levels, an overall sensitivity of 73 and 60%, respectively, was observed. No statistically significant correlation of UGP levels with histologic type, stage, grade, nodal involvement, or bilharzial association was demonstrable, although a trend of increasing mean UGP levels with stage of disease was observed.

The sensitivity of UGP for detecting malignancy in this population of Egyptian bladder cancer patients was comparable to or better than that of other tumor markers. At a specificities of 95% and 100%, sensitivities of 73% and 60% were observed, respectively. UGP showed an extremely low positivity in patients with benign urinary tract disorders.

Due to the extreme difference in UGP levels between patients with benign disease, and bladder cancer patients, UGP is useful for the differential diagnosis of these patients.

The use of UGP is facilitated by the fact that it is a highly stable marker that is measurable in urine, which is a readily obtained and non-invasive sample.

The above example established a level for differentiating bladder cancer in the humans tested for benign urological disease, that is malignant (or bladder cancer) from benign disease. The levels indicate that levels above 0.07 fmol/mL are significant as a distinguishing point with a level above 1.4 fmol/mL defining a clear-cut distinguishing point of malignant cancer herein bladder cancer, over benign disease. Of course, when testing women, there is a chance that urine may indicate the presence of cancer other than bladder cancer. Thus, women who, when tested, test positive, have to be further tested or pretested to rule out other forms of cancer or pregnancy. Biopsy can be used, as can and other known methods for confirming the absence of other cancers or the presence of bladder cancer. Similarly, in pregnancy, in some cases, higher levels of UGP may require further testing to be certain of an indication of bladder cancer. The level for differentiating bladder cancer from benign disease may vary between populations, however, the above example indicates the method for applying the use of UGP to different populations, or additionally with other methods of urine collection such as spot urines that require creatinine or other types of normalization.

The UGP determination can be used as a screening test for bladder cancer. There is a clear distinction between bladder cancer (i.e., malignancy) and benign disease.

Additionally, UGP expression is independent of the histologic type of bladder cancer, therefore, it can be used for the detection, monitoring, or screening of any histotype of disease, especially transitional cell and squamous carcinomas.

In addition to testing for the presence or absence of bladder cancer with a reasonable degree of certainty or as a screening test, the determination of UGP in the urine of males and females can be used to monitor the progression of bladder cancer in an individual known to have bladder cancer. Higher levels of UGP are observed in advanced stages and grades, and stage 1 and 2 disease can be statistically differentiated from stage 3 and 4 disease.

For example, UGP levels can be determined in an individual otherwise known to have bladder cancer. The determination of bladder cancer can be made by the test

of this invention with or without the use of biopsy. Thus, if a first level of UGP at an elevated level is established in a bladder cancer patient, that level can be monitored at 24 hour, 4 day, one week, monthly or other periods to determine the progression or regression of the disease, with higher levels indicating increased cancer and lower levels indicating reduced cancer, and normal levels indicating absence of disease. Normal levels would be considered any level below 0.7 fmol/mL or at least below 1.4 fmol/mL in the urine, when measured in 24 hour urine samples. However, the optimum cutoff for distinguishing normal individuals and individuals with benign disease from individuals with malignancy may need to be determined for each population that is being studied. Other non-blood body fluids such as interstitial fluids and the like, lymph fluids or other fluids found in the urinary tracts of individuals can also be used for determining UGP level.

To monitor the progression or regression of disease in an individual, UGP levels are determined using the Triton® Ciba-Corning kit, as known in the art during preselected time periods, as discussed above. Other assay techniques utilizing monoclonal and/or polyclonal antibodies in sandwich or competitive assay formats can similarly be used to measure UGP levels. In addition, the patient can be treated with a methodology for reducing bladder cancer. Any known methodology can be used as, for example, X-ray treatment, chemical treatments, intravesical chemotherapy, laser therapy, immunotherapy, surgery or the like. After such treatment, the body of the individual can be monitored for UGP levels by monitoring the urine with the levels found again indicating the presence or absence of malignancy even after the treatment. Thus, levels above the levels previously discussed would indicate malignancy. Declining levels would indicate the cancer's decline, while increasing levels would indicate growth and progression of the cancer. Normal levels would indicate probably recovery of the patient.

The monitoring of the patient after treatment with a cancer therapy can be accomplished by carrying out quantitative determination of UGP levels at any selected period desired.



Example 2

A second group of patients, including more with early stage bladder cancer, was evaluated for UGP, with the results appearing in Table VII. UGP levels in stage TI and TII disease were significantly elevated above the normal and control  
5 population and were not significantly different from levels in patients with invasive stage TIII disease. Thus, this data shows that UGP is also a reliable indicator of early stage bladder cancer.

Example 3

10 In a specific example of monitoring the course of cancer in a patient, a patient having bladder cancer at stage T3 is treated by surgery.

The UGP quantitative level in the urine of the patient is determined prior to the treatment at a level of 4.8 fmol/mL. After treatment, the patient is monitored at monthly intervals and found to have levels of 0.3 fmol/mL at 1 month, 2 months, and  
15 3 months post surgery, indicating that the patient was free of residual disease.

In this theoretical example, the patient has declining UGP values to normal, indicating success of the treatment and return of the patient to normal, at least for that period of time. Rising or original values would be an indicator of lack of success of the treatment.

20 However, it should be noted that the quantitative test is indicative for different stages of cancer. Thus, there is a correlation that can be made between UGP levels, and stage 1, stage 2, stage 3, stage 4 or other stages of cancer. The various stages of cancer are known in the art and described in "Clinical Oncology", M.D. Abeloff *et al.*, eds., in the chapter titled *Management of Specific Malignancies, 66 Bladder 1422-*  
25 *1433*, 1995.

While specific embodiments of the present invention have been described above, many variations are possible. In all cases, the invention comprises the determination of UGP levels, to indicate presence or absence of bladder cancer and

distinguish it from benign urinary disease and/or to monitor treatment of patients having bladder cancer.

Table I Expression of UGP in normal and control subjects

Category	N	Mean UGP		Number (%)		exceeding cutoff	Range
		(fmol/mL)	S.D.	<u>0.7 fmol/mL</u>		<u>1.4 fmol/mL</u>	Min. - Max.
Normal <sup>a</sup>	116	0.06	0.15	1 (0.9)		0	0 - 0.8
Benign Urinary tract disease <sup>b</sup>							
Bilharziasis	83	0.13	0.31	6 (7.2)		0	0 - 1.37
Other <sup>c</sup>	14	0.0	N/A	0		0	0 - 0.0
Total benign	97	0.11	0.29	6 (6.2)		0	0 - 1.37

<sup>a</sup> 107 male, 9 female. <sup>b</sup> 90 male, 7 female. <sup>c</sup> Benign prostatic hyperplasia, renal stones, varicocele, bladder ulcer.

Table II Expression of UGP in patients with bladder cancer: breakdown by histology

Category	N	Mean UGP		Number (%) exceeding cutoff		Range
		(fmol/mL)	S.D.	<u>0.7 fmol/mL</u>	<u>1.4 fmol/mL</u>	
SCC	134	4.84	5.40	87 (65)	81 (60)	0 - 20.2
TCC	83	5.40	5.47	59 (71)	52 (63)	0 - 18.5
Other *	20	2.76	3.74	10 (50)	10 (50)	0 - 15.1
Total <sup>b</sup>	237	4.86	5.34	172 (73)	142 (60)	0 - 20.2

\* Adenocarcinoma, undifferentiated carcinoma, verrucous carcinoma, leiomyosarcoma. <sup>b</sup> 171 male, 66 female.

Table III Expression of UGP in patients with bladder cancer: breakdown by stage

Category	N	Mean UGP		Number (%) exceeding cutoff		Range
		( <u>fmol/mL</u> )	S.D.	<u>0.7 fmol/mL</u>	<u>1.4 fmol/mL</u>	
Stage T I&T II	14	3.22	3.91	9 (64)	8 (57)	0 - 12.0
Stage T III	179	4.64	5.40	127 (71)	102 (57)	0 - 20.2
Stage T IV	44	6.24	5.28	36 (81)	32 (73)	0 - 16.5
Total	237	4.86	5.34	172 (73)	142 (60)	0 - 20.2

Table IV Expression of UGP in patients with bladder cancer: breakdown by grade

Category	N	Mean UGP		Number (%) exceeding cutoff		Range
		( <u>fmol/mL</u> )	S.D.	<u>0.7 fmol/mL</u>	<u>1.4 fmol/mL</u>	
Grade 1	41	2.93	4.16	27 (66)	18 (44)	0 - 16.0
Grade 2	118	5.67	5.72	88 (75)	78 (66)	0 - 20.2
Grade 3	78	4.66	5.04	57 (73)	46 (59)	0 - 18.5
Total	237	4.86	5.34	172 (73)	142 (60)	0 - 20.2

Table V Expression of UGP in patients with bladder cancer: breakdown by nodal status and bilharzial association

Category	N	Mean UGP		Number (%) exceeding cutoff		Range
		( <u>fmol/mL</u> )	S.D.	<u>0.7 fmol/mL</u>	<u>1.4 fmol/mL</u>	Min. - Max.
Nodal status						
Negative	205	4.86	5.36	149 (73)	123 (60)	0 - 20.2
Positive	32	4.88	5.21	23 (72)	19 (59)	0 - 17.0
Bilharzial ova in tumor tissue						
Negative	93	4.83	5.46	67 (72)	58 (62)	0 - 19.0
Positive	143	4.82	5.22	104 (73)	83 (58)	0 - 20.2
Total cancer	237	4.86	5.34	172 (73)	142 (73)	0 - 20.2

**Table VI** Expression of UGP in patients with bladder cancer: breakdown by gender

Category	N	Mean age	Mean UGP (fmol/mL)	S.D.
Female	66	50	4.91	5.50
Male	171	53	4.81	5.23



Table VII UGP in the different stages of bladder cancer

UGP (fmol/ml)	T I (n=14)	T II (n=26)	T III (n=188)	T IV (n=49)	T I + II (n=40)	T III + IV (n=237)
Mean	3.42	3.77	4.71	*6.60	3.64	5.1
SE	1.36	1.22	0.39	0.78	0.91	0.35
Median	1.63	1.78	2.15	5.85	1.78	2.60
Minimum	0.34	0	0	0	0	0
Maximum	17.6	30	20.2	17	30	20.2
% with un- detectable levels	21.4	30.8	43.6	63.3	27.5	47.7

\*Significantly different from T III at  $p < 0.05$

CLAIMS

What is claimed is:

1. A test method of determining presence or absence of bladder cancer,  
said method comprising,  
obtaining a non-blood body fluid sample from an individual,  
determining UGP levels in said non-blood body fluid sample of said  
individual with elevated levels above normal indicating a possible cancer of the  
bladder and normal levels indicating the absence of cancer.
2. A method in accordance with claim 1 wherein said non-blood body  
fluid is urine.
3. A method in accordance with the method of claim 2 wherein said urine  
sample is stored at temperatures of up to 37 degrees C for no more than thirty days  
before said determining step.
4. A method in accordance with the method of claim 2 wherein said urine  
sample is stored at a temperature of about minus 20 degrees C for one month prior to  
said determining step without the use of protease inhibitors.
5. A method of determining the presence or absence of bladder cancer in  
accordance with claim 2 wherein said determining step is carried out by a double  
determinant enzyme immunoassay or other methodology.
6. A method of determining possible presence or absence of bladder  
cancer in accordance with claim 2 wherein an individual is first screened to  
determined the absence of other cancers and pregnancy.

7. A method in accordance with the method of claim 2 wherein said elevated level above normal comprises any level above the 90 - 95 percent confidence interval of the normal population, or population with benign disease that are being evaluated.

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8. A method in accordance with the method of claim 2, wherein said elevated level above normal comprises any level above the 100 percent confidence interval of the normal population, or population with benign disease that are being evaluated.

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9. A method of monitoring the effectiveness of a methodology for treating bladder cancer in an individual known to have or to have had bladder cancer, said method comprising, in a first step, measuring the UGP level and treating said individual with a methodology for reducing bladder cancer,

15 and, in a next step, determining UGP level in a non-blood body fluid of said individual with said UGP level indicating a measure of the success of said treatment.

10. A method of claim 9 in which successive samples are evaluated over time to determine the effectiveness of said methodology.

11. A method in accordance with the method of claim 9, wherein said determining step is carried out by a double determinate enzyme immunoassay.

25 12. A method in accordance with the method of claim 9, wherein prior to said treatment, a non-blood body fluid sample from said individual is tested to determine a first UGP level in said non-blood body fluid,

and comparing said next step UGP level with said UGP level determined before said treatment, with the level of UGP after said treatment indicating a measure of success of said methodology.

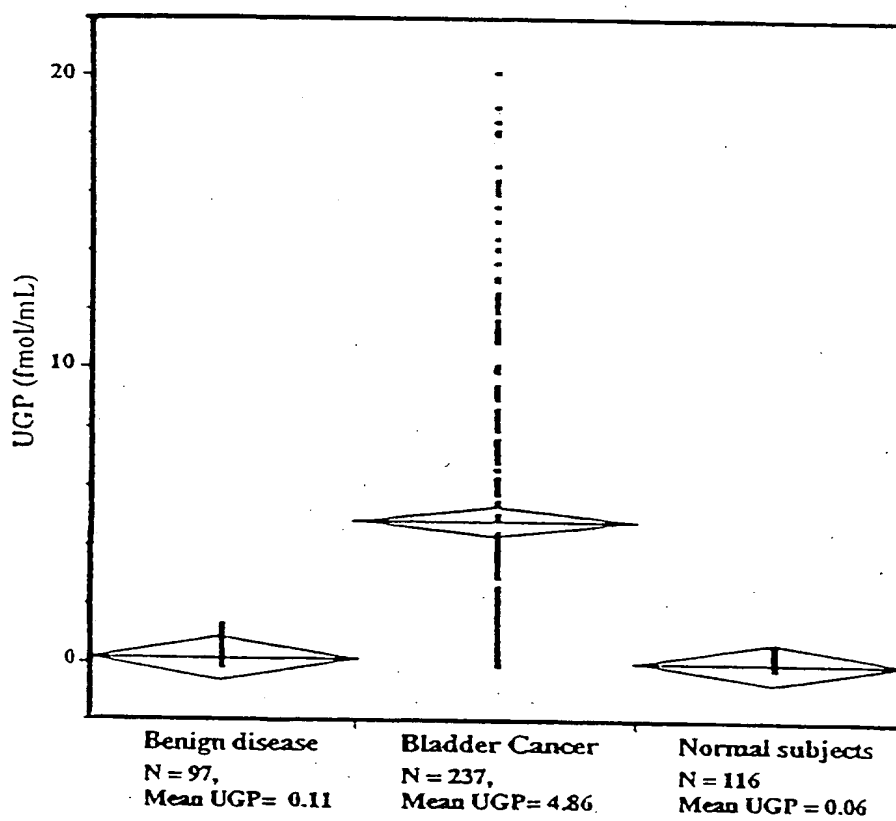
30

13. A method in accordance with the method of claim 9 wherein said non-blood body fluid is urine.

5 14. A method in accordance with the method of claim 11 wherein said non-blood body fluid is urine.

15 15. A method for monitoring the course of bladder cancer in the body, said method comprising determining the presence or absence of  
10 bladder cancer in an individual  
and monitoring said individual to determine the level of UGP in urine of said individual at successive time periods with rising levels indicating increased cancer incidence or disease recurrence and declining levels indicating decreased cancer levels in the bladder.

15 16. A method in accordance with the method of claim 15 wherein said monitoring is carried out in urine samples taken from said individual.



# INTERNATIONAL SEARCH REPORT

International Application No

PC./IB 96/01083

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 G01N33/50 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER RES. (1995), 55(7), 1479-84 CODEN: CNREA8; ISSN: 0008-5472, 1995, XP002022535 NISHIMURA, RYUICHIRO ET AL: "Expression and secretion of the beta. subunit of human chorionic gonadotropin by bladder carcinoma in vivo and in vitro" see abstract see the whole document	1-16
X	US 5 356 817 A (COLE LAURENCE A) 18 October 1994 cited in the application see abstract see claims see column 4, line 28 - line 61	1-16
Y	see column 3, line 48 - line 55	1-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
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- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

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- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- \*Z\* document member of the same patent family

Date of the actual completion of the international search

13 January 1997

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# INTERNATIONAL SEARCH REPORT

International Application No

PC./IB 96/01083

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>CANCER RESEARCH,  vol. 52, no. 14, 15 July 1992,  pages 3901-3907, XP000608910  MARCILLAC I ET AL: "FREE HUMAN CHORIONIC  GONADOTROPIN BETA SUBUNIT IN GONADAL  NONGONADAL NEOPLASMS"  cited in the application  results; last two sentences,  -----</p>	1-16

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC, IB 96/01083

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-5356817	18-10-94	NONE	